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# (54) MODULAR SYSTEM COMPRISING MULTIPLE AUTOMATED MINI-BIOREACTORS FOR HIGH-THROUGHPUT SCREENING (HTS) IN BIOTECHNOLOGY

(57) The invention relates to a method and a device which are used to obtain and maintain multiple biological cultures, with environmental parameters similar to those of large bioreactors /from 1 L) and with a degree of measurement automation similar to that currently obtained using at least the following: measurement and control of temperature, agitation and aeration, measurement and control of pH and dissolved oxygen and measurement of the biomass

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## Description

**[0001]** The present invention relates to a method and a device for obtaining and maintaining multiple biological cultures, with environmental characteristics similar to those of larger bioreactors (from 1 1) and with a degree of measurement automation similar to that currently obtained with microplates (1-2 ml). The environmental characteristics may include temperature measurement and control, stirring and aeration, pH and dissolved oxygen measurement and control and biomass measurement.

## STATE OF THE ART

**[0002]** Development of new or improved organisms, new substances and new processes is being promoted by application of the technology based on the use of genomic and proteomic techniques and combinatorial chemistry. The fields of application of such techniques are as broad as biomedicine, biotechnology, food industry and environment, and without doubt they will be growing in the near future, due to the potential of this technology. One must not forget that many capabilities in the existing organisms are still to discover. In this sense, one will have to face up to the ability to screen, in reasonable time, and with highly technified, automated and standardized means, both the potential of the different cells and natural or modified enzymes and the therapeutic and/or toxic effects of the different molecules developed with said techniques. Systems allowing said screening have been referred to as High Throughput Screening (HTS) systems.

**[0003]** In biotechnology HTS may be dealt at different levels and the existing systems are adapted to each of them according to the specific necessities required by the end user. Therefore, at a molecular level, the interest is focused on permitting multiple assays to be carried out, usually of an enzymatic type, where multiple activities or metabolites are monitored, in different product development stages in biotechnology and more particularly in the field of therapeutic applications. In contrast, at a cellular level, actions are focused on determining, through manual and visual methods, the effect of the product in clinical stages or assays on the effects of several compounds on the cell growth and/or activity.

**[0004]** Technologies currently used in processes for identifying and developing new substances and processes are, on the one hand, small volume culture elements (1-10 ml) which do not allow the *in situ* control of the process critical variables, and which monitoring through conventional techniques is at the very least capital intensive regarding the amount of time required. Furthermore, due to the small reaction volume, the number of samples which can be extracted is reduced. Implementation of *in situ* and in-line measuring systems would therefore involve a great step forward in this field.

**[0005]** On the other hand, the use of in-line measuring systems is limited to bioreactors at a laboratory scale (2-5 liters) and larger scales. However, their high purchasing cost, their operation cost (certainly high if one refers to animal cell cultures) as well as the need for certainly specialized labor makes it difficult the use of bioreactor batteries for studying different culture/reaction conditions for each potential product of interest. Then, there is a potential technological gap that combines the bioreactor measuring and controlling capabilities with the scalability of the small reaction/culture systems. Is this gap that the present patent intends to cover.

**[0006]** In the early stages of development of new substances with biological activity or biotechnological processes, determination of parameters such as for example the media dissolved oxygen concentration, pH, temperature or ionic strength is of special interest. Without a clear knowledge of their values and ranges during the assays, reproducing the ambient conditions in which it was created may result very nearly impossible, making it nearly unfeasible passing to subsequent developing stages. The concept appeal in which this device is based upon is that the final process conditions may be determined in the HTS.

**[0007]** One study of the devices existing in the market reveals that the product having a greater similarity with the one presented, with regard to its design and volume, is a device comprising different vials which potential field of application consists of the synthesis of compounds according to combinatorial chemistry schemes. However, its design is not directed to the use of said vials as bioreactors.

**[0008]** Besides the chemical field, in the medical field there also exist products having certain similarities to the one presented, although in this case the similarities are not as design as functional. Thus, in the hospital field there exist devices based on 1 or 6-well culture plates (culture laboratory typical elements) which are used for maintaining cultures in specified samples of tissue, which is carried out by media continuous perfusion through the culture wells where the tissues are provided. Said plates require, however, a bulky equipment (incubator) for maintaining temperature, moisture and atmospheric composition conditions, and they are devoid of an in-line monitoring system for culture conditions.

## SUMMARY OF THE INVENTION

**[0009]** According to a first aspect, the present invention relates to a device for simultaneously and automatedly carrying out a large number of biological cultures at a small volume and with controlled conditions, in such a way that said conditions are similar to those which can be obtained with large scale bioreactors, which is characterized in that it comprises:

- at least one plate made of a plastic material or other transparent material, which comprises a number of minibioreactors for culture in sterile conditions,

each of them being sealed to the environment, to the rest of the minibioreactors and to a common thermostat bath, each minibioreactor including an individual stirring member for allowing homogenization of its contents, and sterile access points enabling filling, inoculation, gas exchange, liquid exchange and parameter measurement; and

a device intended for receiving at least one plate therein, the plates being fitted through the opening of at least one part of said device, said device comprising means for controlled heat exchange with the thermostat bath, means for energy transmission to the individual stirring members, means for gas exchange through sterile filters and means for non-invasive monitoring and/or controlling of the culture parameters.

[0010] In one preferred embodiment, each plate further comprises a thermostat bath central stirrer.

[0011] Preferably, the plate comprises a number of individual minibioreactors in a polygonal arrangement, with a central area which facilitates common thermostating of said minibioreactors.

[0012] The geometrical arrangement of the wells inside the plate ensures, due to their design, that the overall temperature control may be carried out centrally, thus ensuring the thermal homogenization of all the minibioreactors. Multiple plate lateral coupling is allowed by the polygonal shape with the purpose of forming minibioreactor macro-assemblies which would be fitted out by a common device, with individual or group culture parameter variation.

[0013] According to one embodiment, the device is connected to a computer system, either via a dedicated connection or through a data communication network.

[0014] The system can therefore be indefinitely expanded locally or remotely for achieving the control of a large number of cultures for HTS applications.

[0015] In one embodiment, each minibioreactor comprises an upper cover having an optical port comprising a tube that penetrates within the minibioreactor so that its lower end is immersed into a liquid contained in said minibioreactor.

[0016] The effect of level variations and condensation on the optical measurements carried out through said optical port is thus avoided.

[0017] Optical sensors and light sources may be placed in the optical port at the bottom portion of the plate facing said optical port and/or on the lateral surface of the reservoir.

[0018] The purpose of said sensors is measuring absorption, reflection, scattering or fluorescence of the minibioreactor contents.

[0019] Optical fibers or light guides may be also placed in the optical port at the bottom portion of the plate facing said optical port and/or on the lateral surface of the reservoir.

[0020] The purpose of the optical fibers is remotely measuring absorption, reflection, scattering or fluorescence of the minibioreactor contents.

[0021] In one embodiment, an additional port is fitted through the upper cover so that the end of said port is immersed into the liquid, said end being provided with a filter or semi-permeable membrane.

[0022] This port allows carrying out measurements related to the minibioreactor contents from its outside and without breaking the sterile barrier.

[0023] Other embodiments envisage fitting an additional port through the upper cover so that the end of said port is immersed into the liquid, said end containing sensors or microsensors.

[0024] The sensors or microsensors allow carrying out measurements related to the minibioreactor contents from electrical connections made from its outside.

[0025] According to one embodiment, the heat exchange between the device and the thermostat bath of the plate is carried out by means of a heating resistance that is in contact with a portion of the plate outer perimeter.

[0026] Alternatively, the heat exchange between the device and the thermostat bath of the plate is carried out by means of a Peltier cell that is in contact with a portion of the plate outer surface.

[0027] According to a further embodiment, the heat exchange between the device and the thermostat bath of the plate is carried out by means of a heating resistance that is immersed into said bath.

[0028] In this case, the resistance would be part of the plate itself.

[0029] Other embodiments may envisage that the heat exchange between the device and the bath of the plate is carried out by radio frequency heating performed from the outside.

[0030] The device may comprise a temperature probe immersed into the thermostat bath.

[0031] The probe allows measuring the temperature in said bath and performing the control thereof.

[0032] The device may be further provided with temperature probes immersed into the contents of one or several of the minibioreactors.

[0033] The device may be further provided with an additional minibioreactor surrounded, as in the rest of the minibioreactors, by the thermostat bath, into which a temperature probe is immersed.

[0034] The probe allows measuring the temperature in the bath, with the advantage that the additional minibioreactor may be different in shape and size to the minibioreactors, and being able not to be sterile.

[0035] In one embodiment, the device comprises a stator located below each individual stirring member of the minibioreactor contents for energy transmission to said stirring members.

[0036] Alternatively, the device comprises rotating magnets located below each individual stirring member of the minibioreactor contents for energy transmission to said stirring members, said magnets being rotated by

a mechanical driving system actuated by a common electric motor. According to a further variant, the magnets are rotated by individual electric motors.

[0037] Preferably, the device comprises a stator or a rotating magnet located below the thermostat bath central stirrer for energy transmission to said stirrer.

[0038] In one embodiment, the device comprises means for causing the common thermostat bath stirrer to be rotated from drive sequencing to the minibioreactors.

[0039] Preferably, each minibioreactor has a volume ranging from 5 to 25 ml.

[0040] In one embodiment, the plates are at least in part disposable; they may be formed of a base made of plastic material and at least one cover made of plastic material or other transparent material.

[0041] According to a second aspect, the present invention relates to a method for simultaneously and automatedly carrying out a large number of biological cultures at a small volume and with controlled conditions, in such a way that said conditions are similar to those which can be obtained with large scale bioreactors, which is characterized in that it comprises:

carrying out cultures in sterile conditions in a number of minibioreactors provided at least in one plate of plastic material or another transparent material, said minibioreactors being each sealed to the environment, to the rest of the minibioreactors and to a common thermostat bath, and each minibioreactor including an individual stirring member for allowing homogenization of its contents, and sterile access points enabling filling, inoculation, gas exchange, liquid exchange and parameter measurement;

fitting at least one plate into a device which comprises means for controlled heat exchange with the thermostat bath, means for energy transmission to the individual stirring members, means for gas exchange through sterile filters and means for non-invasive monitoring and/or controlling of the culture parameters; and

non-invasive monitoring and/or controlling of the culture parameters.

## BRIEF DESCRIPTION OF THE DRAWINGS

[0042] For a better understanding of what it has been set forth, drawings are accompanied in which, diagrammatically and only by way of a non-limiting example, a specific embodiment of the invention is herein disclosed. In said drawings:

Figs. 1a and 1b are plan views of respective embodiments of a plate according to the invention containing six minibioreactors, having circular and hexagonal outer profiles, respectively;

Figs. 2a and 2b are vertical sectional views of a plate containing six minibioreactors and the central stirring system supported by the structure or resting on the bottom, respectively;

Figs. 3a and 3b are plan and sectional views, respectively, of one of the wells or minibioreactors;

Figs. 4a and 4b are vertical sectional (4a) and plan views, respectively, of a plate fitted inside a device according to one embodiment of the invention;

Figs. 5a and 5b are perspective views of one embodiment of a device accommodating one plate, which has been depicted with the cover closed and without cover, respectively;

Fig. 6 shows a device according to a further embodiment of the invention accommodating seven plates, with forty-two minibioreactors altogether;

Figs. 7a and 7b are analogous views to Figs. 5a and 5b, for the device in Fig. 6; and

Figs. 8a and 8b show wiring diagrams of the device to a computer and to a computer network, respectively.

## DESCRIPTION OF A PREFERRED EMBODIMENT

[0043] The device according to one embodiment of the invention which is shown in the drawings comprises two clearly differentiated parts:

(A) A closed reservoir 1, made of a plastic material (plate, hereinafter), which features may be seen from Figs. 1a, 1b, 2a and 2b, accommodating a certain number of medium size cells or wells 2 (minibioreactors, hereinafter) (5-25 ml), one of which has been depicted in detail in Figs. 3a and 3b. The plate 1 may be a single-use plate and, once filled with the culture liquids in a sterile environment, it may be handled and conveyed in a non-sterile environment during the rest of the process.

(B) A measuring device or system 3, shown in Figs. 5a, 5b, 7a and 7b, accommodating one or more plates 1 therein and providing the common or individual environmental conditions to the minibioreactors 2. The device 3 also provides the measuring systems allowing automatically tracking and controlling of the suitable parameters for each type of biological culture, and respecting the sterile barrier in the plate 1. The device 3 may be replicated indefinitely by means of its connection to a computer network with the purpose of performing, controlling and monitoring a large number of assays, with variation in its reaction/growth conditions and with repetition of conditions in groups of minibioreactors 2 or plates 1, as necessary.

[0044] The different parts of the device and its operation are fully described below.

Description of the plate

[0045] As shown in Figs. 1a and 1b, a plate 1 may contain six minibioreactors 2 and a central stirring system 4.

[0046] The central symmetry of the plate 1 is to be emphasized which allows maintaining the wells 2 at the same temperature with only one central heater 5 heating a common thermostat bath 6, stirred by said stirring system 4. Said bath 6 is filled through a plug 7, which may be also located on an upper cover (not shown).

[0047] In Fig. 1a the plate has a circular outer profile, while in the variant of the embodiment shown in Fig. 2b the profile is hexagonal in shape. The perimeter may be of different shapes; the polygonal shape allows several plates 1 to be laterally fitted forming a multiple-well compact assembly 2 (forty-two in the case of six-well hexagonal plates).

[0048] In the vertical sectional view in Fig. 2a a plate 1 may be seen having a central stirring system 4 that is supported by the structure; the figure shows the minibioreactors or wells 2 laterally surrounded by the thermostat bath 6, the central stirrer 4 and upper and lower accesses 8 through which heat exchange is supplied, as it will be disclosed further on, to the bath 6 and energy to the central stirrer 4.

[0049] In one variant shown in Fig. 2a, the stirring system 4 rests on the bottom of the plate 1, and there is only provided one upper access 8.

[0050] Figs. 3a and 3b show in greater detail one of the wells or minibioreactors 2, in plan view and in vertical sectional view.

[0051] The minibioreactor 2 comprises an optical port 21, which allows carrying out measurements by transmission or reflection through its lower window 22, which is kept always immersed below the level of the liquid 23, thus providing an optical path which length is not level-dependent.

[0052] The minibioreactor 2 is likewise provided with gas inlet and outlet tubes 24, a septum 25 for filling, inoculating and eventually supplying and draining liquids, an initially not perforated optional port 26, which would allow non-optical probes or micro-probes to be added, and a rotor 27 of a magnetic stirrer.

[0053] In short, the plate accommodates, as stated, a certain number of minibioreactors (for example, six), said minibioreactors being each sealed to the environment, to the rest of the minibioreactors and to a bath permitting common thermostating thereof. The central symmetry geometrical arrangement of the wells inside the plate ensures, due to their design, that the overall temperature control may be carried out centrally, thus ensuring the thermal homogenization of each the minibioreactors. Each minibioreactor includes an individual stirring member for allowing homogenization of its contents. The energy required for imparting the movement to said element will be provided contactlessly from the outside. The minibioreactors will be further provided with access ports for gas inflow and outflow with the purpose of providing the culture with the suitable oxygen partial pressure as well as other gases as necessary. Sterility of said access will be ensured by filters connected to said ports. Filling, inoculation and, in case of being necessary, sample collection from minibioreactors will be carried out by a third port through a septum-type closure. For continuous or feedbatch processes said septum may be used for inserting cannulae for supplying and draining liquids throughout growth.

[0054] Culture parameter measurements will be carried out from the outside, without interfering the plate sterile barrier. Possible measuring methods contemplated herein are as follows:

- optical access to the contents of each minibioreactor, from the walls thereof or from transparent portions therefrom.
- electric access to the sensor or micro-sensor outer contacts provided inside the minibioreactors before the sterilization process.
- measurements carried out from the outside on liquids subjected to ionic exchange with the bioreactor contents through membranes maintaining the sterile barrier.

[0055] The volume of the plate corresponding to the outside of the minibioreactors will be filled with a liquid (for example, water) not necessarily sterile. Stirring of such liquid will be imparted from the outside in a like manner to the stirring of the bioreactor contents. Heat will be supplied to or drained from said liquid, from the outside as well, for obtaining the thermal homogenization of the bath and the well contents.

[0056] The plate as a whole, or at the very least, a part thereof that will be in contact with the culture media, will be disposable and it will be presented, already sterilized, inside a bag or on compartments, with which it becomes a consumable product for the user. Its filling, inoculation, and connection to the filters does no require any other tool other than a laminar flow chamber and they are the only processes to be performed in a sterile area.

Description of the device

[0057] A plate 1 as noted above fitted inside a device 3 is shown in Figs. 4a and 4b in a vertical sectional view and in a plan view; this device has sub-systems supplying the plate (for delivering energy and heat on the plate, as well as measuring and control systems) and is, in turn, housed in an outer box 9.

[0058] The outer box 9 may accommodate the different parts of the device 3 and the plate 1 of minibioreactors 2, as well as a data control and acquisition system (not shown in Figs. 4a, 4b), although this may be also located outside the box 9 and connected thereto by cables.

[0059] As the cover of the outer box 9 closes, alignment and connection of the different sub-systems in the plate 1 is facilitated.

[0060] The different shading patterns in Figs. 4a, 4b allow the sub-systems of the device 3 to be identified which provide the heat exchange to the thermostat bath 6 of the plate 1 and the energy to the central stirrer 4 of said bath and to the stirrers 27 in each well 2, and to the optical sensors (lower part of the plate 1 and optical port 21) or other type of sensors of micro-sensors. Fig. 4b shows how the spacing of the different sub-systems in circular crowns is facilitated by the well central symmetry arrangement.

[0061] The outer shape of the device 3 is depicted, in a possible variant of one embodiment, in Fig. 5a: it comprises a base 31 in which a plate 1 is housed (Fig. 5b), that is closed by a cover 32.

[0062] As it may be seen from the plan view of Fig. 6, a device may be also designed accommodating several plates 1 having a polygonal perimeter: the figure shows an arrangement of seven plates 1 (forty-two minibioreactors altogether) inside a single device. The device perimeter may be, in this case, hexagonal 3' or circular 3".

[0063] In general, providing m plates 1 having a polygonal perimeter each having n wells, macro-plates having n x m wells 2 may be thus formed.

[0064] Wells 2 in each plate 1 will be at the same temperature, so that the system allows obtaining m experiments at different temperatures each having n replicas.

[0065] Figs 7a and 7b are analogous to Figs. 5a and 5b, for one embodiment of the device 3 with seven plates 1 each having six wells 3.

[0066] In short, the outer measuring device or system will accommodate one or more plates therein, which will be fitted through the aperture of one or more portions of said device. Its functions will be as follows:

- Controlled heat exchange with the bath thermostating the n minibioreactors simultaneously.
- Energy transmission to the n individual stirrers.
- Gas exchange through the sterile filters with culture aeration through the head or by means of a tube that penetrates inside the liquid. Aeration in each well will be able to be controlled separately.
- Non-invasive monitoring and/or controlling of the culture parameters. By way of examples, pH, optical density (cell concentration) and oxygen dissolved separately in each well.
- Possibility of supplying and draining of liquids.
- Possibility of providing further probes and biosensors.
- Possibility of system customization for specific applications.

Connection to a control and operating system

[0067] In Fig. 8a, a device 3, containing a single plate or a set of m plates is connected to a computer or a control system 10 which will manage the subsystem setup and control parameters and collect the measurement data. The connection between both is carried out either by a cable 11 which is adapted to a data transmission standard protocol or by means of a custom-made connection which contemplates both data and signal transmission.

[0068] In fig. 8b, a series of devices 3, containing any combination of plates or set of plates, is connected to a computer o control system 10 which will manage the setup and control parameters and collect the measurement data. The connection between both is carried out either by cables 12 connecting the systems to a data communication network 13, with the possibility of incorporating remote devices and/or computers through a connection 14 to an external network 15.

[0069] In short, the device will be able to be connected to a computer system, through a dedicated connection o through a data communication network. The control software will allow connection of a sufficient number of devices such that it provides automatic control capability on the large number of cultures necessary for the application of the HTS system.

[0070] The managing system of the whole bioreactor assembly would fix the control instructions of each unit and monitor the evolution of the parameters of each bioreactor and its response to the possible stimuli applied. The proposal then consists of the application of the capabilities and versatility of a device that comprises a battery of small volume minibioreactors made of plastic pieces and provided with micro-probes for simultaneously carrying out multiple tests in the development of biotechnological processes and products. One important aspect is that the device is of the modular type, that is, the plates comprising n minibioreactors will be able to be multiplied m times depending on the number of tests and the rate at which they have to be carried out in the final application.

[0071] The measurement of the biological and enzymatic activities themselves, created by genetic modifications or induced by different substances is thus of a great importance for informing and assessing the object being researched and establishing corresponding correlations regarding activities, properties and possibilities of application of the different exploitable substances or processes at industrial level. It is worth mentioning that the more relevant biological variables on which the culture tracking in the proposed device will be focused are the measurement of the cell concentration (by means of light absorption or refraction optical systems) and the measurement of the cell activity (from measurements of the oxygen consumption). The measuring system of both variables will allow keeping track of the response of the cultures at different varying conditions of the physicochemical environment of the organisms under culture and the disturbances therein by adding substances having physiological effects, either growth-enhancing effects or, on the contrary, with toxic effects for

culture growth. Likewise, the system will also allow the possibility of keeping track of enzyme-catalyzed reactions, produced both by cells harvested in the system under different controlled conditions and enzymes or multi-enzymatic systems in in vitro conditions. In addition, the system will be able to include all those measuring and control elements necessary for culture growth in optimum conditions (pH, temperature, dissolved oxygen concentration, stirring, conductivity and osmolarity.

## Claims

1. Device for simultaneously and automatedly carrying out a large number of biological cultures at a small volume and with controlled conditions, in such a way that said conditions are similar to those which can be obtained with large scale bioreactors, **characterized in that** it comprises:

   at least one plate (1) made of a plastic material or other transparent material, which comprises a number of minibioreactors (2) for culture in sterile conditions, each of them being sealed to the environment, to the rest of the minibioreactors (2) and to a common thermostat bath (6), each minibioreactor (2) including an individual stirring member (27) for allowing homogenization of its contents, and sterile access points (21, 24, 25, 26) enabling filling, inoculation, gas exchange, liquid exchange and parameter measurement; and
   a device (3) intended for receiving at least one plate (1) therein, the plates (1) being fitted through the opening of at least one part of said device (3), said device (3) comprising means for controlled heat exchange with the thermostat bath (6), means for energy transmission to the individual stirring members (27), means for gas exchange through sterile filters and means for non-invasive monitoring and/or controlling of the culture parameters.

2. Device as claimed in claim 1, **characterized in that** each plate (1) further comprises a thermostat bath central stirrer (4).

3. Device as claimed in claims 1 or 2, **characterized in that** the plate (1) comprises a number of individual minibioreactors (2) in a polygonal arrangement, with a central area which facilitates common thermostating of said minibioreactors (2).

4. Device as claimed in any of claims 1 to 3, **characterized in that** the device (3) is connected to a computer system (10, 15), either via a dedicated connection or through a data communication network

5. Device as claimed in any of the preceding claims, **characterized in that** each minibioreactor (2) comprises an upper cover having an optical port (21) including a tube that penetrates within the minibioreactor so that its lower end (22) is immersed into a liquid (23) contained in said minibioreactor (2).

6. Device as claimed in claim 5, **characterized in that** optical sensors and light sources are placed in the optical port (21), at the bottom portion of the plate (1) facing said optical port (21) and/or on the lateral surface of the reservoir.

7. Device as claimed in claims 5 or 6, **characterized in that** optical fibers or light guides are placed in the optical port (21), at the bottom portion of the plate facing said optical port (21) and/or on the lateral surface of the reservoir.

8. Device as claimed in any of claims 5 to 7, **characterized in that** an additional port (26) is fitted through the upper cover so that the end of said port is immersed into the liquid (23), said end being provided with a filter or semi-permeable membrane.

9. Device as claimed in any of claims 5 to 8, **characterized in that** an additional port (26) is fitted through the upper cover so that the end of said port is immersed into the liquid (23), said end containing sensors or microsensors.

10. Device as claimed in any of claims 1 to 9, **characterized in that** the heat exchange between the device (3) and the thermostat bath (6) of the plate (1) is carried out by means of a heating resistance that is in contact with a portion of the plate outer surface.

11. Device as claimed in any of claims 1 to 9, **characterized in that** the heat exchange between the device (3) and the thermostat bath (6) of the plate (1) is carried out by means of a Peltier cell that is in contact with a portion of the plate (1) outer surface.

12. Device as claimed in any of claims 1 to 9, **characterized in that** the heat exchange between the device (3) and the thermostat bath (6) of the plate (1) is carried out by means of a heating resistance that is immersed into said bath (6).

13. Device as claimed in any of claims 1 to 9, **characterized in that** the heat exchange between the device (3) and the bath (6) of the plate (1) is carried out by radio frequency heating performed from the outside.

14. Device as claimed in any of claims 1 to 13, **characterized in that** it comprises a temperature probe immersed into the thermostat bath (6).

15. Device as claimed in any of claims 1 to 14, **characterized in that** it comprises temperature probes immersed into the contents (23) of one or several of the minibioreactors (2).

16. Device as claimed in any of claims 1 to 15, **characterized in that** it comprises an additional minibioreactor surrounded, as in the rest of the minibioreactors (2), by the thermostat bath (6), in which a temperature probe is immersed.

17. Device as claimed in any of claims 1 to 16, **characterized in that** it comprises a stator located below each individual stirring member (27) of the minibioreactor (2) contents, for energy transmission to said stirring members (27).

18. Device as claimed in any of claims 1 to 16, **characterized in that** it comprises rotating magnets located below each individual stirring member (27) of the minibioreactor (2) contents, for energy transmission to said stirring members (27), said magnets being rotated by a mechanical driving system actuated by a common electric motor.

19. Device as claimed in any of claims 1 to 16, **characterized in that** it comprises rotating magnets located below each individual stirring member (27) of the minibioreactor (2) contents, for energy transmission to said stirring members (27), said magnets being rotated by individual electric motors.

20. Device as claimed in any of claims 2 to 19, **characterized in that** it comprises a stator or a rotating magnet located below the thermostat bath (6) central stirrer (4), for energy transmission to said stirrer (4).

21. Device as claimed in claim 2 and any of claims 17 to 19, **characterized in that** it comprises means for causing the common thermostat bath (6) stirrer (4) to be rotated from the drive sequencing to the minibioreactors (2).

22. Device as claimed in any of the preceding claims, **characterized in that** each minibioreactor (2) has a volume ranging from 5 to 25 ml.

23. Device as claimed in any of the preceding claims, **characterized in that** the plates (1) are at least in part disposable.

24. Device as claimed in any of the preceding claims, **characterized in that** the plates (1) are formed of a base made of plastic material and at least one cover made of plastic material or other transparent material.

25. Method for simultaneously and automatedly carrying out a large number of biological cultures at a small volume and with controlled conditions, in such a way that said conditions are similar to those which can be obtained with large scale bioreactors, **characterized in that** it comprises:

carrying out cultures in sterile conditions in a number of minibioreactors (2) provided at least in one plate (1) of plastic material or another transparent material, said minibioreactors (2) being each sealed to the environment, to the rest of the minibioreactors (2) and to a common thermostat bath (6), and each minibioreactor (2) including an individual stirring member (27) for allowing homogenization of its contents, and sterile access points (21, 24, 25, 26) enabling filling, inoculation, gas exchange, liquid exchange and parameter measurement;
fitting at least one plate (1) into a device (3) which comprises means for controlled heat exchange with the thermostat bath (6), means for energy transmission to the individual stirring members (27), means for gas exchange through sterile filters and means for non-invasive monitoring and/or controlling of the culture parameters; and
non-invasive monitoring and/or controlling of the culture parameters.

Figure 1a

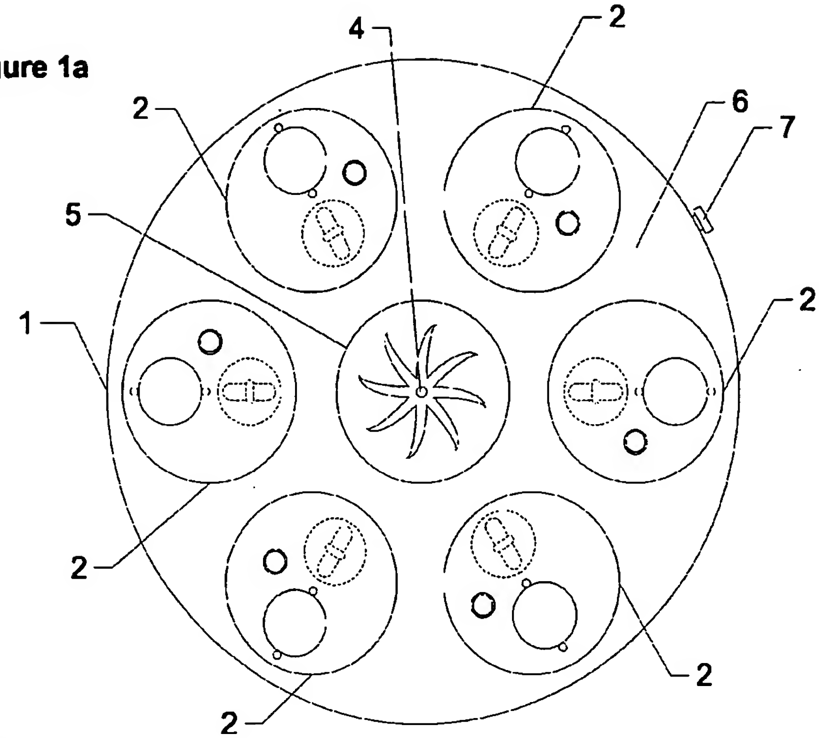



Figure 1b

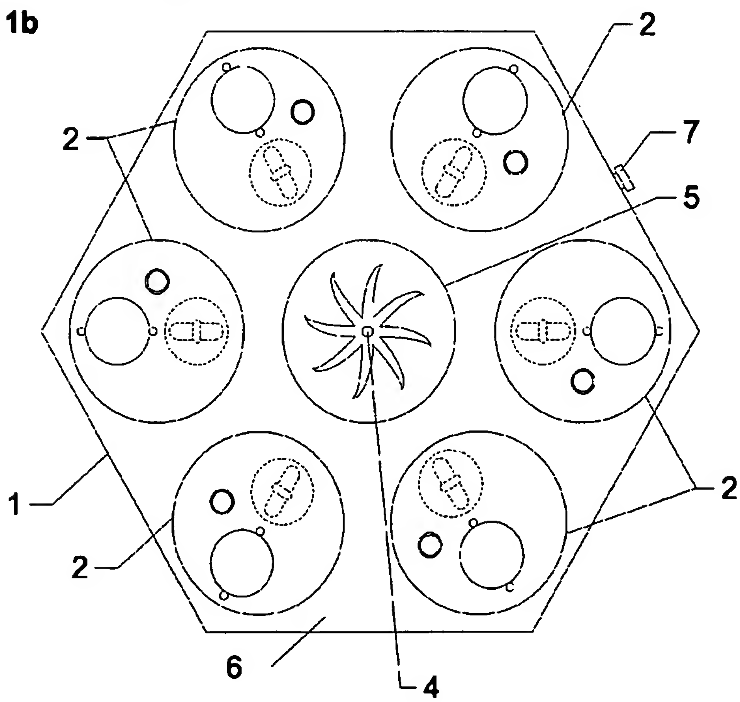


Figure 2a

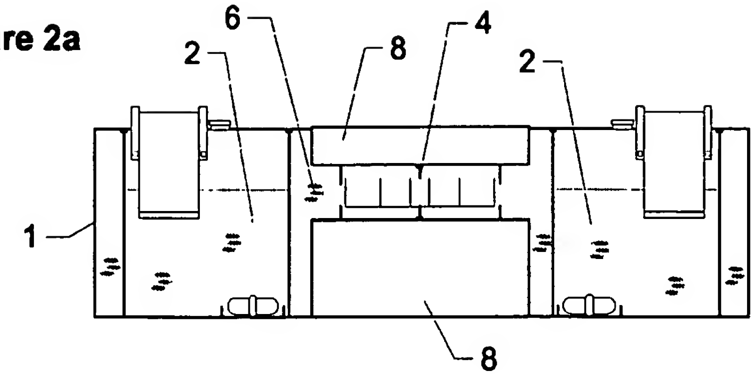



Figure 2b

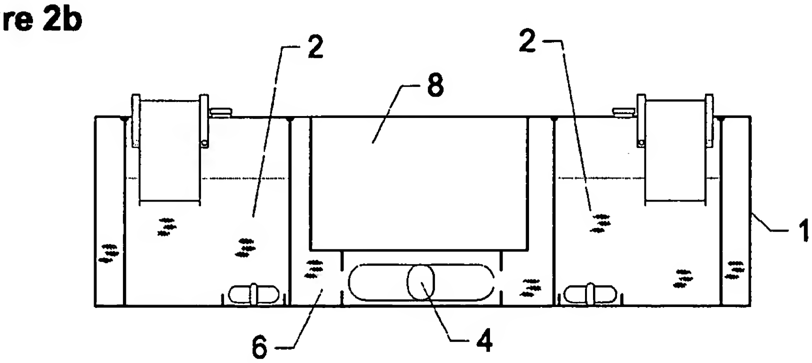



Figure 3a

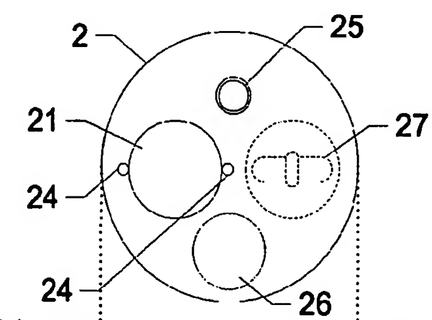



Figure 3b

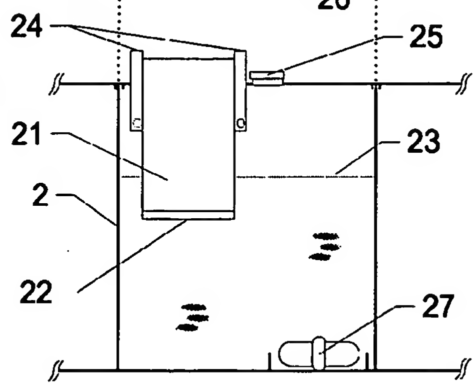
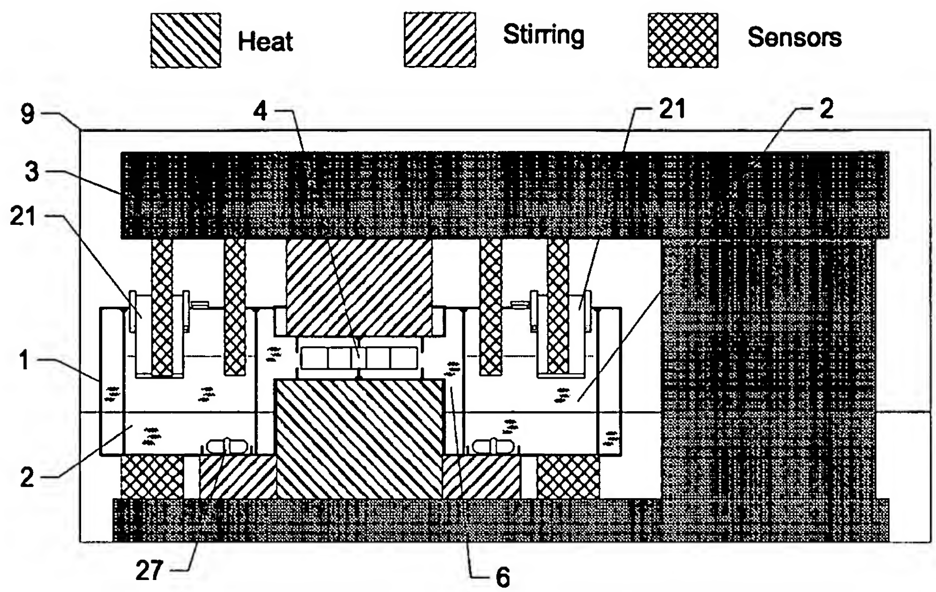


Figure 4a



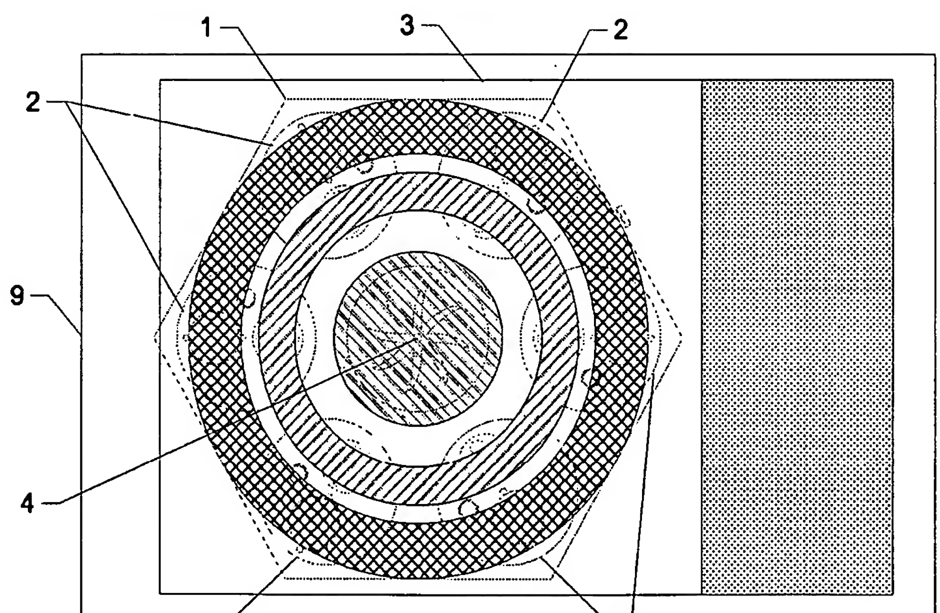




Figure 4b

Figure 5a

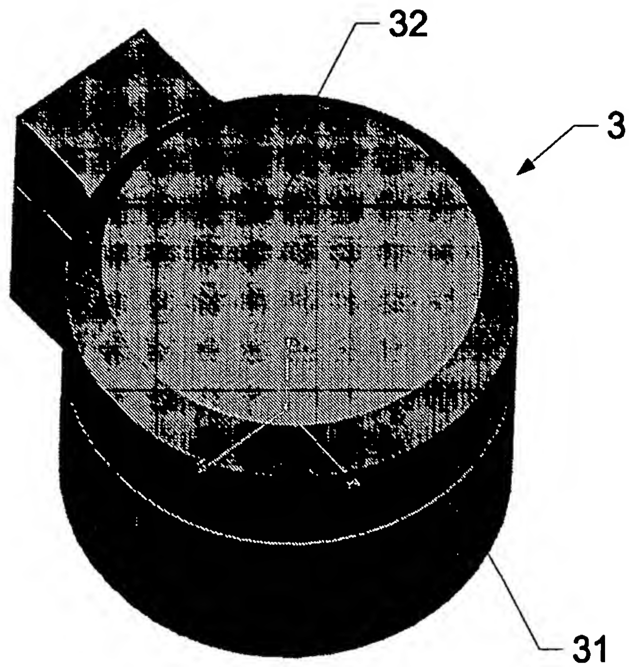



Figure 5b

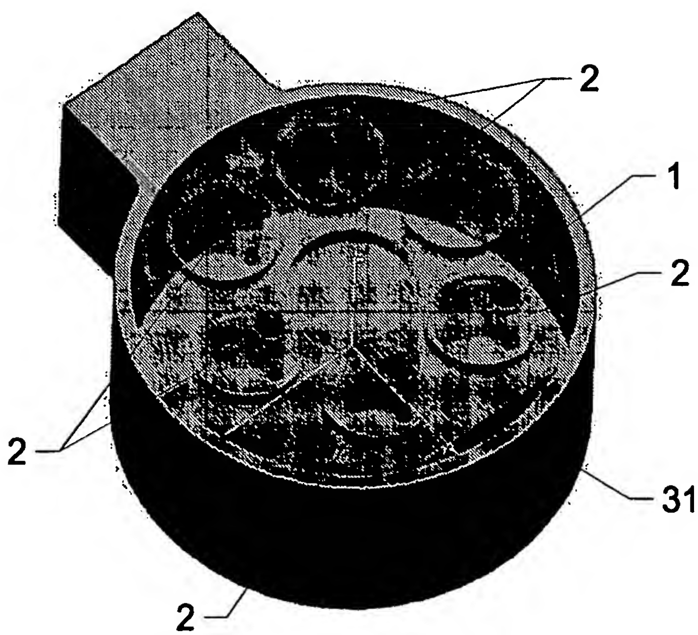


Figure 6

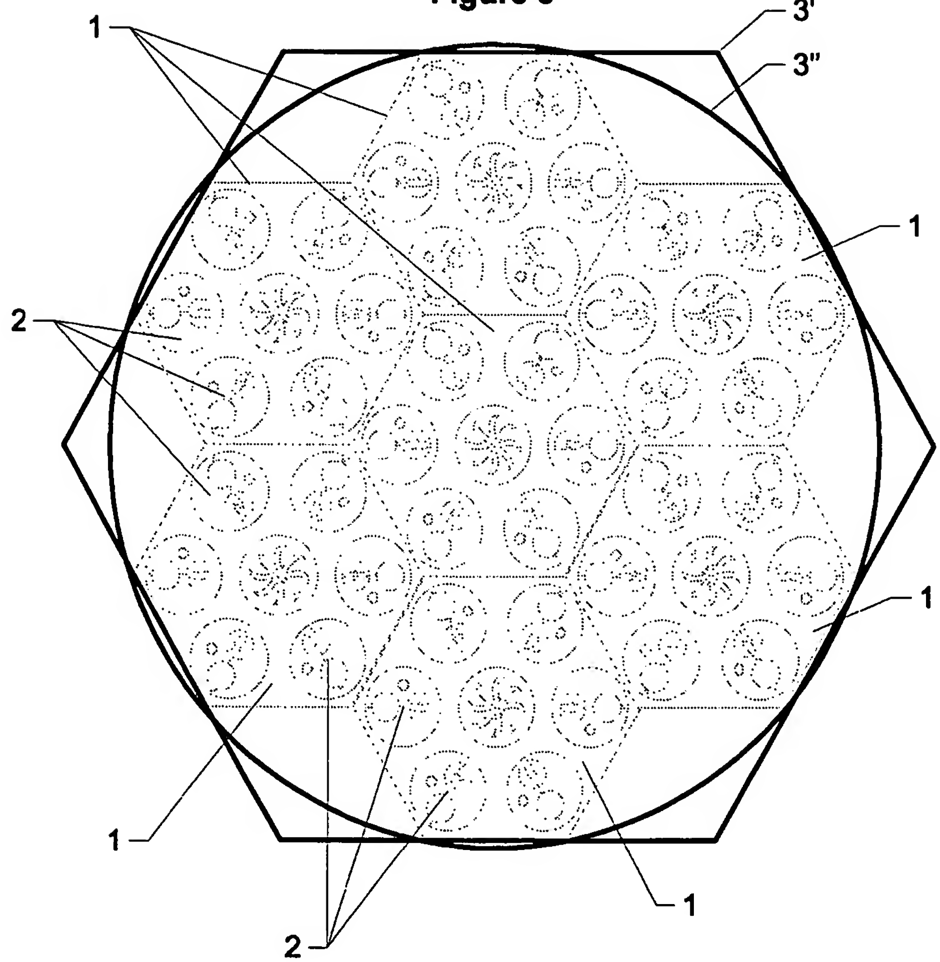


Figure 7a

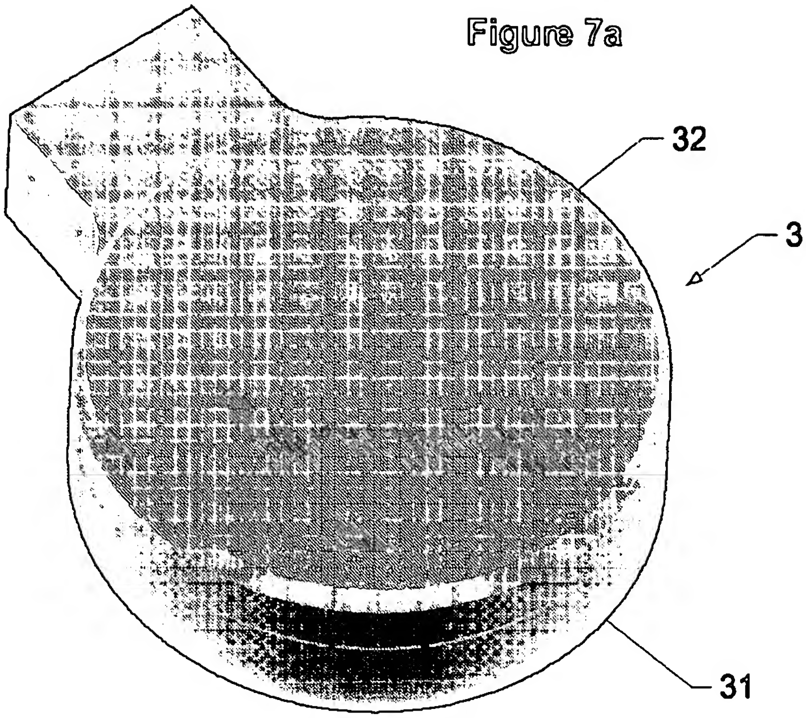



Figure 7b

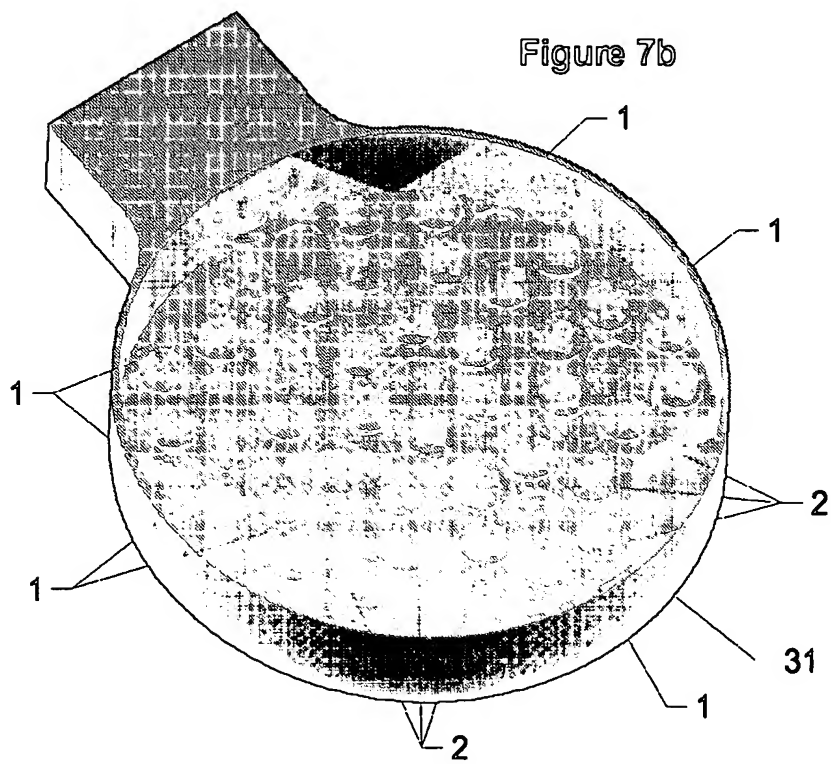
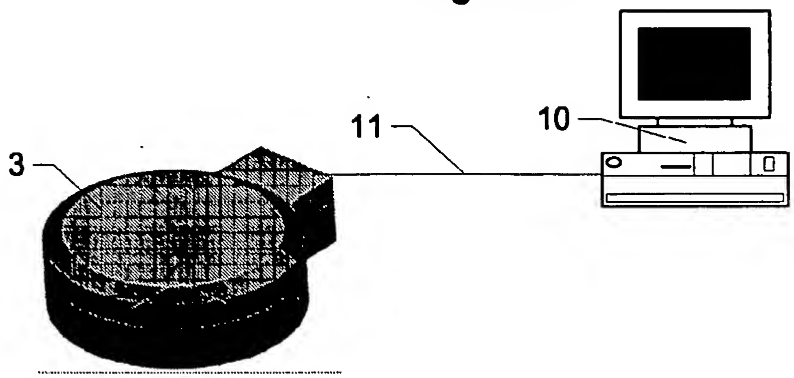


**Figure 8a**



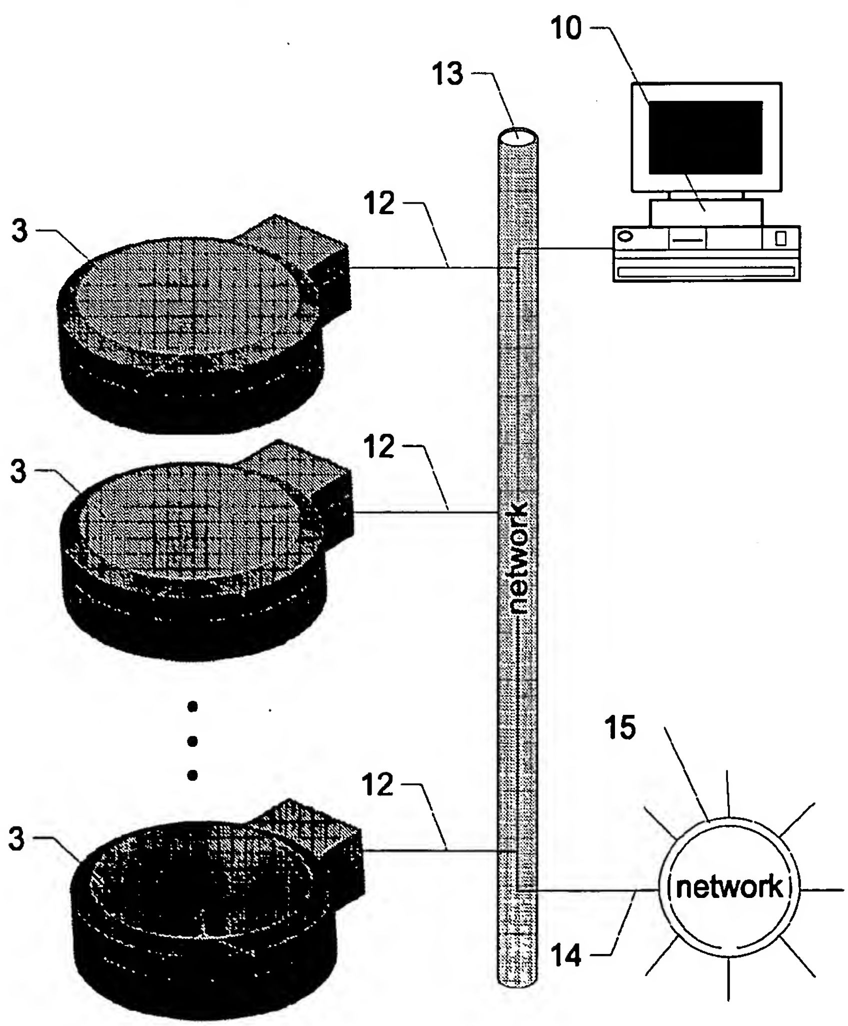




**Figure 8b**

## INTERNATIONAL SEARCH REPORT

International application No.
PCT/ ES 2003/000607

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12M1/34, C12M1/36, C12M1/02, C12M3/00, C12Q1/00, G01N33/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12M, C12Q, G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DWPI, EPODOC, PAJ, CIBEPAT, LATIPAT.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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| Y | DE 19710652 A (Jungkeit) 17.9.1998<br>Column 1, lines 5-14; Column 1, line 53 - column 3, line 40 | 1,2,4-9,14-16,22-25 |
| Y | WO 2000/044876 A (Institut für Chemo- und Biosensorik Münster) 03.08.2000.<br>Claims | 1,2,4-9,14-16,22-25 |
| A | WO 2002/083852 A (Bioprocessors Corporation) 24.10.2002<br>page 2, line 13-page 3, line 5; page 3, line 16- page 6, line 21 | 1, 4-9,14-16,22-25 |
| A | US 2002/0146816 A (Vellinger et al.) 10.10.2002<br>The whole document | 1, 4-9,14-16,22-25 |

[x] Further documents are listed in the continuation of Box C. [x] See patent family annex.

* Special categories of cited documents:
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"O" document referring to an oral disclosure, use, exhibition or other means
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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 02 MARCH 2004 (02.03.04) | 16 MARCH 2004 (16.03.04) |
| Name and mailing address of the ISA/<br>S.P.T.O.<br>Facsimile No. | Authorized officer<br><br>Telephone No. |

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/ES 2003/000607

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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| A | FR 2831182 A (Université de Nantes) 25.04.2003<br>Claims | 1, 5-7 |
| A | WO 2000/045953 A (BILATEC Gesellschaft zur Entwicklung Biotechnologischer Systeme) 10.08.2000<br>Claims 1, 12, 13, 21; page 12, lines 4-29 | 1, 10, 11 |

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